

WHAT IS CLAIMED IS:

1. A composition of matter comprising a fluorescent product derived from a modified form of an *Aequorea* wild-type GFP polypeptide, characterized in that upon oxidation and cyclization of amino acid residues in the modified form corresponding to positions 65 to 67 of wild-type GFP polypeptide sequence [SEQ ID NO:2] a product exhibiting a different excitation and/or emission spectrum from a corresponding product derived from the wild-type GFP polypeptide sequence is formed.
2. A composition according to claim 1, wherein the product exhibits an alteration in the ratio of two main excitation peaks relative to the corresponding product derived from wild-type GFP.
3. A composition according to claim 2, wherein increased fluorescence is exhibited at a shorter-wavelength peak of the two main excitation peaks.
4. A composition according to claim 3, wherein the modified form of the wild-type GFP sequence comprises a replacement of Ser at a position corresponding to position 202 in the wild-type GFP sequence by Phe and a replacement of Thr at a position corresponding to position 203 by Ile.
5. A composition according to claim 2, wherein increased fluorescence is exhibited at a longer-wavelength peak of the two main excitation peaks.
6. A composition according to claim 5, wherein the modified form of the wild-type GFP sequence comprises a replacement of Ile at a position corresponding to position 167 of the wild-type GFP sequence by Val or Thr.

7. A composition according to claim 5, wherein the modified form of the wild-type GFP sequence comprises a replacement of Ser at a position corresponding to position 65 of the wild-type GFP sequence by Thr, a replacement of Met at position 153 with Ala, and a replacement of Lys at position 238 with Glu.

8. A composition according to claim 1, wherein the product fluoresces at a shorter wavelength than the corresponding product derived from wild-type GFP.

9. A composition according to claim 8, wherein the modified form of the wild-type GFP sequence comprises a replacement of Tyr at a position corresponding to position 66 of the wild-type GFP sequence by Phe, His or Trp.

10. A composition according to claim 8, wherein the modified form of the wild-type GFP sequence comprises a replacement of Tyr at a position corresponding to position 66 of the wild-type GFP sequence by His and a replacement of Tyr at position 145 with Phe.

11. A composition according to claim 8, wherein the modified form of the wild-type GFP sequence comprises a replacement of Tyr at a position corresponding to position 66 of the wild-type GFP sequence by Trp, a replacement of Asn at position 146 by Ile, a replacement of Met at position 153 by Thr, a replacement of Val at position 163 by Ala, and a replacement of Asn at position 212 by Lys.

12. A composition according to claim 8, wherein the modified form of the wild-type GFP sequence comprises a replacement of Tyr at a position corresponding to position 66 of the wild-type GFP sequence by Trp, a replacement of Ile at position 123 by Val, a replacement of Tyr at position 145 by His, a replacement of His at position 148 by Arg, a replacement of Met at position 153 by Thr, a replacement of Val at position 163 by Ala, and a replacement of Asn at position 212 by Lys.

13. A composition according to claim 1, wherein ~~the~~ ^{the} product exhibits enhanced emission relative to the corresponding product derived from wild-type GFP.

14. A composition according to claim 13, wherein the modified form of the wild-type GFP sequence comprises a replacement of Ser at a position corresponding to position 65 of the wild-type GFP sequence by an amino acid selected from the group consisting of Ala, Cys, Thr, Leu, Val and Ile.

15. A composition according to claim 14, wherein the amino acid is Cys or Thr.

16. A substantially pure oligonucleotide sequence encoding a modified form of an *Aequorea* wild-type GFP polypeptide sequence according to any one of claims 1-15.

17. A method for monitoring expression of a gene encoding a polypeptide, comprising:

forming a combined sequence comprising the gene and an oligonucleotide sequence according to claim 16, in which combined sequence both the gene and the oligonucleotide sequence are in the same reading frame; and observing for fluorescence characteristic of a product derived from a polypeptide sequence encoded by the oligonucleotide sequence, the fluorescence indicating expression of a fusion protein encoded by the combined sequence.

18. A method for simultaneously monitoring expression of a first gene and a second gene in a single cell, tissue or organism, the first gene encoding a polypeptide different from a polypeptide encoded by the second gene, said method comprising:

forming a first combined sequence comprising the first gene and a first oligonucleotide sequence according to claim 16, in which first combined sequence

both the first gene and the first oligonucleotide sequence are in the same reading frame;

forming a second combined sequence comprising the second gene and a second oligonucleotide sequence selected from the group consisting of an oligonucleotide sequence encoding a wild-type GFP and an oligonucleotide sequence according to claim 16 different from the first oligonucleotide sequence, in which second combined sequence both the second gene and the second oligonucleotide sequence are in the same reading frame; and

observing for fluorescence characteristic of products derived from polypeptide sequences encoded by the first and second oligonucleotide sequences, the fluorescence indicating expression of fusion proteins encoded by the combined sequences.

19. A method according to claim 18, wherein agents are screened for utility in activating expression of at least one of the first and second genes.
20. A method according to claim 18, wherein locations of proteins expressed from the first and second genes are compared.
21. A method according to claim 18, wherein temporal sequence of expression of the first and second genes is determined.
22. A method for monitoring transcription of a gene, comprising:
forming a combined sequence comprising upstream regulatory elements of the gene and an oligonucleotide sequence according to claim 16, in which combined sequence both the upstream regulatory elements and the oligonucleotide sequence are in the same reading frame; and
measuring the fluorescence characteristics of a product derived from a polypeptide sequence encoded by the oligonucleotide sequence, the fluorescence

indicating transcription of the oligonucleotide under the control of the upstream regulatory elements of the gene.

23. A method for simultaneously monitoring expression of a first gene and a second gene in a single cell, tissue or organism, the first gene encoding a polypeptide different from a polypeptide encoded by the second gene, said method comprising:

forming a first combined sequence comprising upstream regulatory elements of the first gene and a first oligonucleotide sequence according to claim 16, in which first combined sequence both the upstream regulatory elements of the first gene and the first oligonucleotide sequence are in the same reading frame;

forming a second combined sequence comprising the upstream regulatory elements of a second gene and a second oligonucleotide sequence selected from the group consisting of an oligonucleotide sequence encoding a wild-type GFP and an oligonucleotide sequence according to claim 16 different from the first oligonucleotide sequence, in which second combined sequence both the upstream regulatory elements of the second gene and the second oligonucleotide sequence are in the same reading frame; and

observing for fluorescences characteristic of products derived from polypeptide sequences encoded by the first and second oligonucleotide sequences, the fluorescences indicating transcription under the control of the respective upstream regulatory elements of the first and second genes.